Study of Antibacterial Activities of Carbon Nanotube Based Epoxy Composites to Prevent Biofouling in Marine Environment: A Lab Scale Approach

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Abstract

The multiwalled carbon nanotubes were reinforced in 0.25 wt percentage to prepare epoxy composite. The CNT reinforced polymer sample and neat epoxy samples were exposed to pure culture of bacteria such as *Bacillus* and *Pseudomonas* to test the inhibition of microbial growth on the composites. Biofilm characterization and microscopic studies showed excellent performance on the composite samples, as well as a reduced microbial growth with 0.25 wt% of the MWCNTS in the polymer.

Keywords

CNT; Antibacterial; Composites; Bacillus; Pseudomonas

Introduction

Biofouling is one of the major concerns for the naval structures. These structures are affected by fouling of various kinds like deposition or particulate, scaling or crystallization, microbiological, debris or macro-fouling, corrosion and corrosion products. The main goal of this study is to device a method which will reduce the biofouling in naval structures and improve its resistance, as well to improve the antimicrobial properties of naval structures using nanotechnology. Biofilm are formed as a result of the bacteria present in water. The growth of biofilm is inversely proportional to the water flow rate/flow velocity. Water is a good insulator of heat and biofouling leads to loss in heat transfer since the biofilms entrap water in them.

Therefore, enhancing the antimicrobial properties of the naval structures is vital for the efficient functioning of the marine equipment.

Effective long term antifouling protection for navy ships and submerged equipments may be realized in the mid 1970's (E. J. Dyckman, 2009). Navy makes increasing use of polymers for relatively simple parts, such as load-bearing polymeric matrix composites, special coatings for signature control, coatings for corrosion reduction in waste-holding tanks, fuel storage tanks, and metal pipe linings, hoses, pipes, and gaskets. Light weight, corrosion-resistance, and ease of manufacture provide the main impetus for the use of polymers in these applications, and the market share of polymers for such applications is anticipated to continue to grow steadily in the future.

Biofouling incurs many disadvantages and undesirable effects to surfaces. Bacterial adhesion has been associated with interference in medical materials and devices, (M. Katsikogianni, 2004, G. Harris, 2006). In the marine environment, biofouling raises costs through increased fuel consumption owing to a larger drag on ships and ferries (Adkins 1990, Brady 2000, Callow 2002). For naval structure, biofouling causes increased hydrodynamic drag, resulting in increased fuel consumption and decreased speed and range.

The present principal control of biofouling is available for mechanical and chemical treatment (T. Vladkova 2007), but all this is not very fruitful. The surface engineering of polymers to control biofouling is a new approach to control biofouling on naval structures. Usually, the surface modifications alter the surface morphology, topography and roughness. These polymer may be toxic for bacteria but can extend the life time of the naval structure.

The synthesized polymers may have better characteristics compared to conventional ones because of high strength and stiffness/weight ratio, corrosion-resistance, manufacturing flexibility, chemical stability and low cost. Polymers like epoxy holds the capability

of the resistance to biofouling. Therefore, nanocomposite coating will be on naval structures and enhance its antibacterial properties by reducing adhesion.

Nano reinforcement enhances physical and chemical properties of the polymer compared to the micro reinforcements. The nanoparticles have large surface to volume fraction and depend on their shape, size and composition. Therefore, the goal of this work is to synthesize CNT based epoxy nanocomposite coating on the naval material to increase its antibacterial efficiency.

Materials and Methods

Filler

Multiwalled Carbon Nanotubes (MWCNTs) have been synthesized by Chemical Vapour Deposition (Camphor as carbon source) method used as filler for the composite. The tube diameter ranges from 5-10 nm and length of 1 micron. The filler loading is 0.25 wt%.

Matrix

The epoxy resin Araldite LY-556 (diglycidyle ether of bisphenol) was mixed with an amine type hardener (HY 951) under the weight ratio of 17:1. Epoxy resins have been produced from a reaction between epichlorohydrin and bisphenol-A.

Synthesis of Epoxy Based CNT Composite Coating

MWCNTs were dispersed with acetone 1 hr at 2000 RPM at Room temperature (RT) and solution was dispersed with resin and stirred for 1 hr at 80°C at 2000 RPM. The mixture was stirred for 1 hr at 2000 RPM. After addition of hardener, the mixture was stirred for 15 minutes at 2000 RPM. The mixture was cured in vacuum oven at 120^{o} C for 7 hrs to get the composites.

Laboratory Exposure Studies of Pure Cultures of Bacteria

Neat epoxy and CNT reinforced epoxy sample were tested for antibacterial properties. The pure culture of gram positive (*Bacillus*) and gram negative (*Pseudomonas*) bacterial culture was prepared by using nutrient broth (Hi-media, M002). The samples was tied in nylon thread and exposed to these cultures for 72 hours, 15 days and 30 days. After the mentioned period, in the bacterial culture, a set of sample was used to estimate the Total viable count (TVC) of bacteria. The sample was taken out from the culture and sonicated with 15 ml of sterile phosphate buffer (0.0425 g KH_2PO_4, 0.19 g $MgCl_2$ per litre). Serial dilutions of the bacterial cell suspension were prepared and 0.1 ml of each dilution was plated onto nutrient agar bought from Hi Media, M001.

The plates were incubated for 24–48 h at 32^{o}C and the total viable count (TVC) was estimated APHA (1989). Statistical analysis of the data was carried out using MYSTAT Software. Three replicates of two sets were analyzed for each experimental condition. A Student T-test was performed to assess significance in the difference between bacterial counts on neat epoxy and CNT reinforced epoxy sample.

Microscopy Characterization of Biofilm

For epifluorescence microscopy, the method was followed as Direct Acridine orange (DAOC) study. The other set of specimens of each condition was stained with 0.1% acridine orange solution for 30 min and rinsed with deionized water to remove excess stain. The stained specimens were observed under an epifluorescence microscope (Nikon Eclipse 80i Epifluorescence Microscope, excitation filter BP 490; Barrier Filter O 515) (Mah TC, 2001). Acridine orange, a fluorescent dye, differentially stains single stranded RNA and double stranded DNA, fluorescing orange when intercalated with former and green while complexing with the latter. Its active living cells and biofilm will be fluorescent orange.

Results

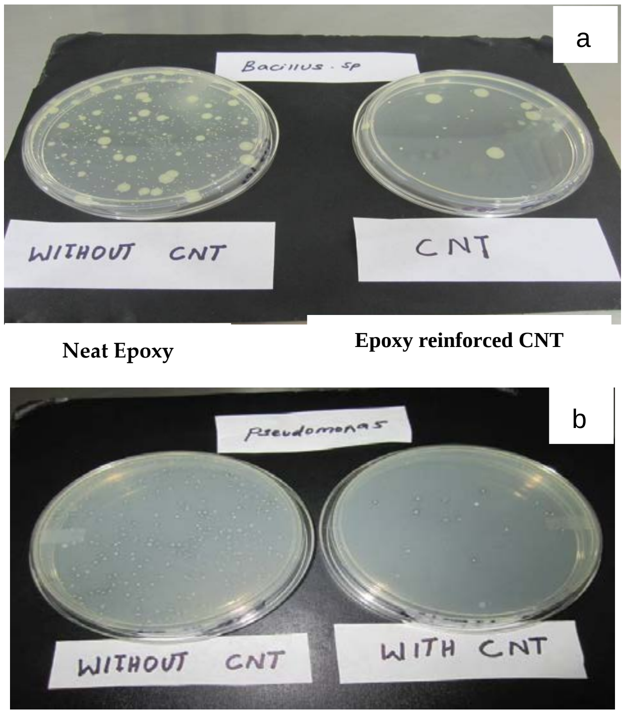
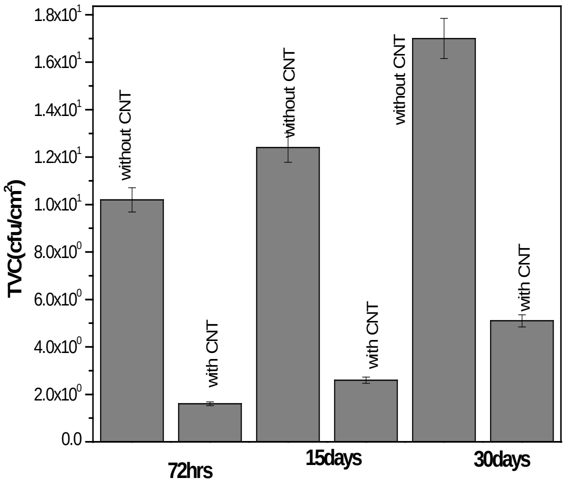
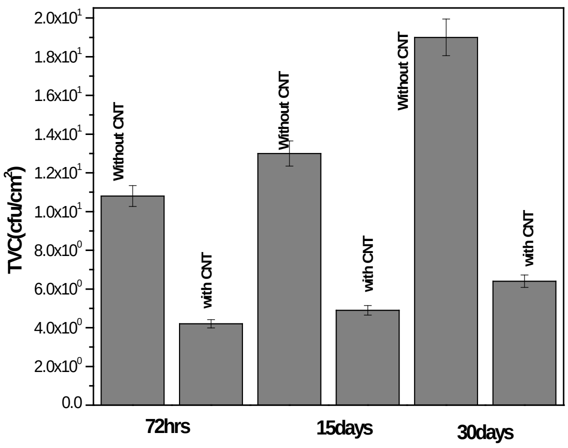


FIGURE 1 (A) TVC PLATES FOR *BACILLUS* SPS. (B) TVC PLATES FOR *PSEUDOMONAS* SPS

The significant decrease in bacterial counts on Neat epoxy sample compared with CNT reinforced epoxy sample as shown in Figure 1 (a & b) & Graph 1 & 2. Results of TVC were expressed as colony forming units (cfu/cm²). CNT reinforced epoxy sample showed remarkable reduction compared to neat epoxy samples in both gram positive and gram negative bacteria. The number of the bacterial colony was more in *Pseudomonas* culture compared to *Bacillus* culture.



GRAPH:-1-TVC FOR PURE CULTURE OF GRAM POSITIVE BACTERIA (*BACILLUS* SPS.)



GRAPH:-2-TVC FOR PURE CULTURE OF GRAM NEGATIVE BACTERIA (*PSEUDOMONAS* SPS.)

Epifluorescence micrographs showed significant decrease in bacterial attachment after 30 days of exposure observed between neat epoxy and CNT reinforced epoxy in both the culture. The numbers of cells which were attached and fluorescing was seen to be very high in case of *Pseudomonas* culture (Figure 2).

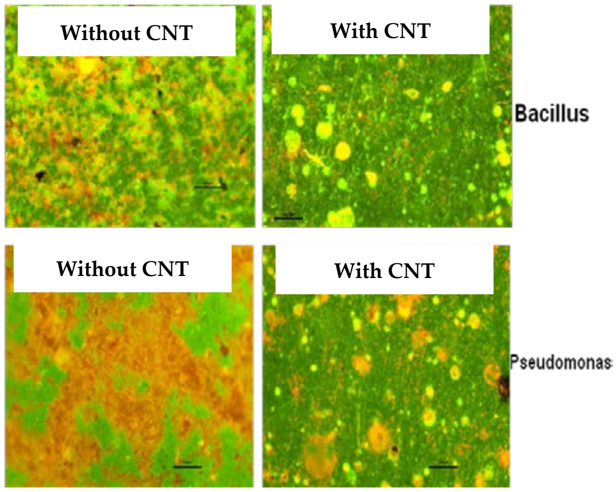


FIGURE 2 EPIFLUORESCENCE MICROGRAPHS OF NEAT EPOXY AND CNT REINFORCED EPOXY SAMPLE FOR 30DAYS

Discussion

Fiber reinforced composite materials have been the alternatives to metal, steel and wood in many applications because of their low cost to weight savings, improved life cycle, high specific tensile and compressive strength, good fatigue and corrosion resistant. The use of GRFP/CRFP is hindered due to non-availability of experimental data and durability in the marine environment.

The advantage of polymer nanocomposites in Naval sector is light weight structure, resulting in the reduction in the fuel consumption and the emission of CO_2, increment in the speed of the naval ships, less maintenance cost of marine vessels. The major concern of marine fouling raises the fuel cost by 30% and thus a coating that resists fouling should be developed. Comparative analysis of existing materials like Fiber Reinforced Polymers gets more weight, less mechanical strength, low strength at low temperature and as well processing is not very easy. On the other side, when compared with Epoxy CNT Composites, it shows good properties with resistance to microbial growth. So, this study was planned for MWCNT based epoxy nanocomposite for the naval material to check its antibacterial efficiency. A short term exposure study for 72h, 15 days and 30 days was planned in the pure culture of gram positive and gram negative bacteria. The comparative analysis of gram positive and gram negative showed that the growth of gram negative was more pronounced compared to gram positive. These studies aimed at the antimicrobial properties of the nanocomposite coating over the neat polymer samples. The polymer based nanocomposite may attain good biofouling resistance and can be used

for shipping industry and marine industry.

ACKNOWLEDGEMENT

The bio-lab & Epifluorescence Microscope (Nikon-Eclipse 80i) facilities of Centre for Nanoscience & Nanotechnology @ Sathyabama University is deeply acknowledged.

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